Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (original) A method of culturing peripheral lymphoid organ cells comprising:

culturing peripheral lymphoid organ cells on a three-dimensional scaffolding which is covered or surrounded with culture medium under conditions effective to generate and maintain mature and functional peripheral lymphoid organ cells, wherein said three-dimensional scaffolding allows cells in the culture medium to have cell to cell contact in three dimensions.

2. (original) The method according to claim 1, wherein the scaffolding is selected from the group consisting of tangled fibers, porous particles, sponge, sponge-like material, and combinations thereof.

3. (original) The method according to claim 1, wherein the scaffolding is formed from a material selected from the group consisting of metal, glass, ceramic, plastic, hydroxyapatite, treated or untreated bone, a synthetic polymer, a natural substance, a semisynthetic material, and combinations thereof.

4. (original) The method according to claim 3, wherein the material is degradable.

5. (original) The method according to claim 3, wherein the material is non-degradable.

6. (original) The method according to claim 1, wherein the culture medium contains exogenous growth factors, cytokines, lymphokines, hormones, chemokines, interleukins, mitogens, antigens or antigenic fragments thereof, or combination thereof.

7. (original) The method according to claim 6, wherein the culture medium contains cytokines which are selected from the group consisting of interleukin-2, interleukin-4, interleukin-6, interleukin-10, interleukin-7, interleukin-12, flt-3 Ligand, stem cell factor, thrombopoietin, CD40 ligand, BAC-1, L-BCGF, soluble interleukin 6R, and combinations thereof.

8. (original) The method according to claim 6, wherein the culture medium contains an antigen which is selected from the group consisting of a peptide, protein, carbohydrate, glycoprotein, proteoglycan, lipopolysaccharide, nucleic acid, virus, cells, cell fragment, tissue, and combination thereof.

9. (original) The method according to claim 8, wherein the antigen is a tumor antigen.

10. (original) The method according to claim 6, wherein the culture medium contains antigens and antigenic fragments that are presented by antigen presenting cells.

11. (original) The method according to claim 10, wherein the antigen presenting cells are dendritic cells.

12. (original) The method according to claim 6, wherein the culture medium contains antigens or antigenic fragments that are present as a conjugate.

13. (original) The method according to claim 1, wherein the peripheral lymphoid organ cells are B-cells, T-cells, or combination thereof.

14. (original) The method according to claim 13, wherein the peripheral lymphoid organ cells are T-cells which are selected from the group consisting of cytotoxic T-cells, helper T-cells, and combination thereof.

15. (original) The method according to the claim 13, wherein the peripheral lymphoid organ cells are B-cells which are selected from the group consisting of immature B cells, naïve B cells, memory B-cells, B1 cells, B2 cells, plasma cells, and combination thereof.

16. (original) The method according to claim 1, wherein the peripheral lymphoid organ cells are selected from the group consisting of natural killer (NK) cells, dendritic and follicular dendritic cells, granulocytes, macrophages, and stromal cell subsets.

17. (original) The method according to claim 1, wherein the cultured peripheral lymphoid organ cells are selected from the group consisting of spleen cells, lymph node cells, thymus cells, Peyer's patches cells, and combinations thereof.

18. (original) The method according to claim 17, wherein the cultured peripheral lymphoid organ cells are spleen cells.

19. (original) The method according to claim 1, wherein the cultured peripheral lymphoid organ cells express a surface marker selected from the group consisting of CD5, CD23, CD69, CD25, MHC class I or II, CD80/86, CD138, CD38, CD27, CD8, CD4, CD3, CD45-RO, CD45-RA, and combinations thereof.

20. (original) The method according to claim 1, wherein the cultured peripheral lymphoid organ cells fail to express a surface marker selected from the group consisting of CD5, CD23, CD69, CD25, MHC class I or II, CD80/86, CD138, CD38, CD27, CD8, CD4, CD3, CD45-RO, CD45-RA, and combinations thereof.

21. (original) The method according to claim 1, wherein said culturing is carried out without addition of external mitogens.

22. (currently amended) The method according to claim 1 further comprising:

~~seeding or reseeding the culture medium with peripheral lymphoid organ cells,~~ adding peripheral lymphoid cells, primary lymphoid organ cells, stem cells, or combinations thereof to the culture medium covering or surrounding the three-dimensional scaffolding.

23. (currently amended) The method according to claim 1 ~~22, wherein the culture medium is reseeded with~~ further comprising:

adding additional peripheral lymphoid organ cells selected from the group consisting of spleen cells, lymph node cells, Peyer's patches cells, and combinations thereof to the culture medium covering or surrounding the three-dimensional scaffolding.

24. (original) The method according to claim 1, wherein said culturing is carried out with an antigen or antigenic fragment thereof in the culture medium and under conditions effective to produce antigen-specific lymphocytes.

25. (original) The method according to claim 24, wherein said culturing is carried out under conditions effective to permit clonal selection, expansion, and/or affinity maturation of lymphocytes.

26. (original) The method according to claim 24, further comprising:
adding an adjuvant to the cell culture.

27. (original) The method according to claim 24, wherein the antigen is selected from the group consisting of a peptide, protein, carbohydrate, glycoprotein, proteoglycan, lipopolysaccharide, nucleic acid, virus, cells, cell fragment, tissue, and combinations thereof.

28. (original) The method according to claim 24, wherein the antigen is a tumor antigen.

29. (original) The method according to claim 24, wherein the antigen or antigenic fragment are presented by antigen presenting cells.

30. (original) The method according to claim 24, wherein the antigen or antigenic fragment are present as a conjugate.

31. (withdrawn) The method according to claim 24, wherein said culturing is carried out under conditions effective to permit the antigen-specific lymphocytes to produce antibodies.

32. (withdrawn) The method according to claim 24 further comprising:
immortalizing the antigen-specific lymphocytes.

33. (withdrawn) The method according to claim 32, wherein the lymphocytes are B-cells, T-cells, or combinations thereof.

34. (withdrawn) The method according to claim 32, wherein said immortalizing is induced.

35. (withdrawn) The method according to claim 34, wherein said immortalizing comprises:

fusing the antigen-specific lymphocytes to a cell line under conditions effective to produce a hybridoma cell line.

36. (withdrawn) The method according to claim 35 further comprising:

recovering monoclonal antibodies from the hybridoma cell line.

37. (withdrawn) The method according to claim 32, wherein said immortalizing is spontaneous.

38–120. (canceled)

121. (new) The method according to claim 1, wherein the scaffolding comprises openings that the peripheral lymphoid organ cells are able to enter.

122. (new) The method according to claim 121, wherein the openings are from about 15 microns to about 1000 microns.

123. (new) The method according to claim 122, wherein the openings are from about 100 microns to about 300 microns.

124. (new) The method according to claim 1, wherein:

the method further comprises isolating the peripheral lymphoid organ cells from a peripheral lymphoid organ before said culturing; and

the peripheral lymphoid organ cells comprise lymphocytes.